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## Ultrastructural features of primate granule cell bodies show important differences from those of rats: axosomatic synapses, somatic spines and infolded nuclei

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Granule cells of the primate dentate gyrus were examined in the electron microscope where they displayed significantly less axosomatic synapses than granule cells in rodents. In addition, primate granule cells frequently had infolded cell nuclei and somal spines which are features that are both rare in rodents. Since the granule cell body is an important site for  $\gamma$ -aminobutyric acid (GABA)ergic inhibitory control, the reduced number of axosomatic synapses in monkeys suggests that local inhibitory connections of primate granule cells are less than that of rodents. Together, these differences may indicate that the primate granule cells are physiologically more active than rat granule cells.

Granule cells of the dentate gyrus are regarded as a cell type with small variability in their morphological features in various species<sup>6</sup>. However, recent studies of the monkey dentate gyrus have shown that primate granule cells display significant variability in light microscopic preparations<sup>10,12</sup>. This variability includes granule cells with basal dendrites as well as differences in the length of apical dendrites and spine density.

In contrast to the light microscopic differences, the ultrastructure of granule cells has been found to be similar in rats and monkeys. Thus, granule cells display a round cell nucleus of about 10–12  $\mu$ m in diameter, a thin rim of perikaryal cytoplasm and both symmetric and asymmetric axosomatic and axodendritic synapses<sup>5,10,14</sup>. However, the analysis of monkey granule cells<sup>10</sup> used exclusively Golgi-electron microscopic preparations and was limited to a sample of only 10 neurons. The Golgi-electron microscopic preparations do not allow for a detailed quantitative analysis of synapses because gold particles often obscure labeled synapses and the membranes are usually not preserved as well as that found in routine electron microscopic preparations. Therefore, the previous description of granule cells<sup>10</sup> simply stated that asymmetric and symmetric axosomatic synapses were found but no attempt was made to determine their frequency.

In the present study, we wanted to determine the frequency of axosomatic synapses for primate granule cells and the relative numbers of symmetric and asym-

metric synapses. Many of the terminals that form symmetric axosomatic synapses are derived from  $\gamma$ -aminobutyric acid (GABA)ergic basket cells<sup>9,10</sup>. The proportion of GABAergic inhibitory neurons in the granule cell layer of monkeys is less than that of rats<sup>1</sup>. Furthermore, the relative numbers of basket cells to granule cells is also much less for monkeys than rats<sup>11,13</sup>. These data suggested that the inhibitory input to primate granule cells may be different than that for rats.

Two young, adult Rhesus monkeys (*Macaca mulatta*) were used in this experiment. The animals were transcardially perfused with a solution containing 4.0% paraformaldehyde, 1.25% glutaraldehyde, 0.002% calcium chloride in a 0.12 M phosphate buffer (pH 7.2). The brains were removed from the skull and kept overnight in the refrigerator before dissecting the hippocampus on the following day. Blocks of tissue obtained from the middle level of the main body of the hippocampus were processed for electron microscopy using a routine schedule that included post-staining with 2% OsO<sub>4</sub> and embedding in Medcast. Serial thin sections were obtained from the blocks. Formvar-coated slot grids (1  $\times$  2 mm) were used because they facilitated the examination of large areas of the granule cell layer in a single thin section. The sections were stained with uranyl acetate and lead citrate and examined with a Philips CM-10 electron microscope. The quantitative analysis of axosomatic synapses of granule cells used a goniometer stage

to tilt the grids for the identification of synapses and their type. Synapses were classified as symmetric or asymmetric using the established criteria of postsynaptic density, width of synaptic cleft and the shape of synaptic vesicles<sup>14</sup>.

The granule cell layer of the primate dentate gyrus is densely packed with granule cells. Axon terminals formed both asymmetric (Fig. 1A) and symmetric (Fig. 1B) synapses with the somata of granule cells. Granule cells at the border with the molecular layer had slightly more axosomatic synapses than the granule cells at the hilar border. However, in both cases the number of axosomatic synapses was surprisingly low. The mean value of axosomatic synapses per granule cell per thin section was  $0.67 \pm 0.04$  (S.E.M.). Although the number of synapses found on a single granule cell body per thin section ranged from 0 to 4, many granule cells did not display a single synapse (Fig. 2A). Although both types of synapse were found, the number of asymmetric axosomatic synapses was very small, amounting to about 3% of all axosomatic synapses of granule cells (Table I).

The nuclei of granule cells were round or ovoid and they often displayed small finger-like indentations (Fig.

2A). The frequency of these infoldings of nuclear membrane was greater for the granule cells that formed the border with the molecular layer (40%) than for those located deeper inside the granule cell layer or at the hilar border (18%).

Somal spines were commonly found for granule cells (Fig. 2B,C). They varied greatly in diameter and length. Also, they displayed a continuous membrane with the cell body but lacked polyribosomes and granular endoplasmic reticulum. The spines contained a fine, flocculent material that was clearly different from the cytoplasmic matrix (Fig. 2B,C). In this analysis, 239 granule cells from the entire depth of the granule cell layer were examined and 64 of them (27%) displayed a total of 89 spines. This reflected the fact that the number of spines for a single granule cell varied from one to 3. Only 4 of the 89 spines were observed to form synapses with axon terminals (Fig. 2B).

The main finding of the present study is that primate granule cells display a much lower number of symmetric axosomatic synapses per granule cell per thin section than rat granule cells. It is interesting to note that primate granule cells display about 25% of the number of

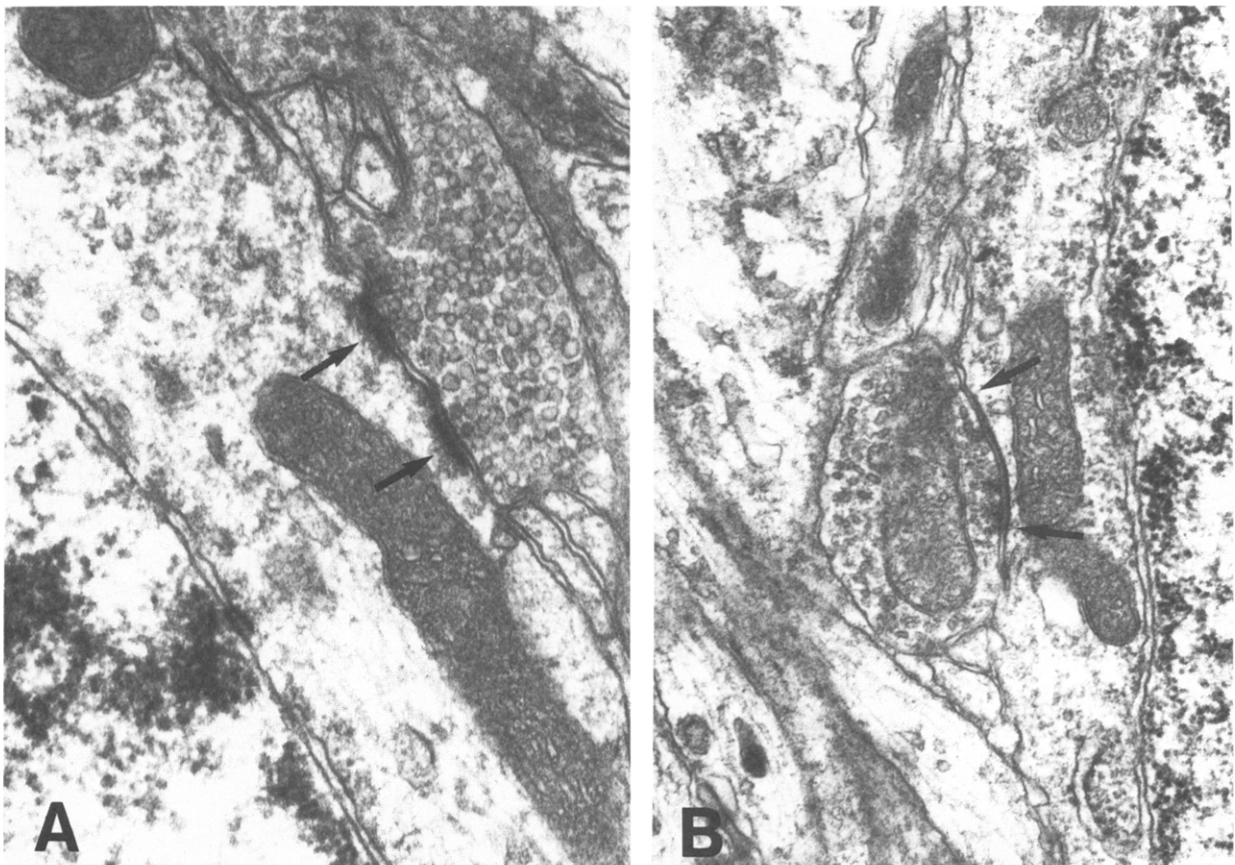


Fig. 1. Electron micrographs of axon terminals that form asymmetric (A) and symmetric (B) synapses (arrows) with the somata of granule cells. A,  $\times 56,000$ ; B,  $\times 39,000$ .

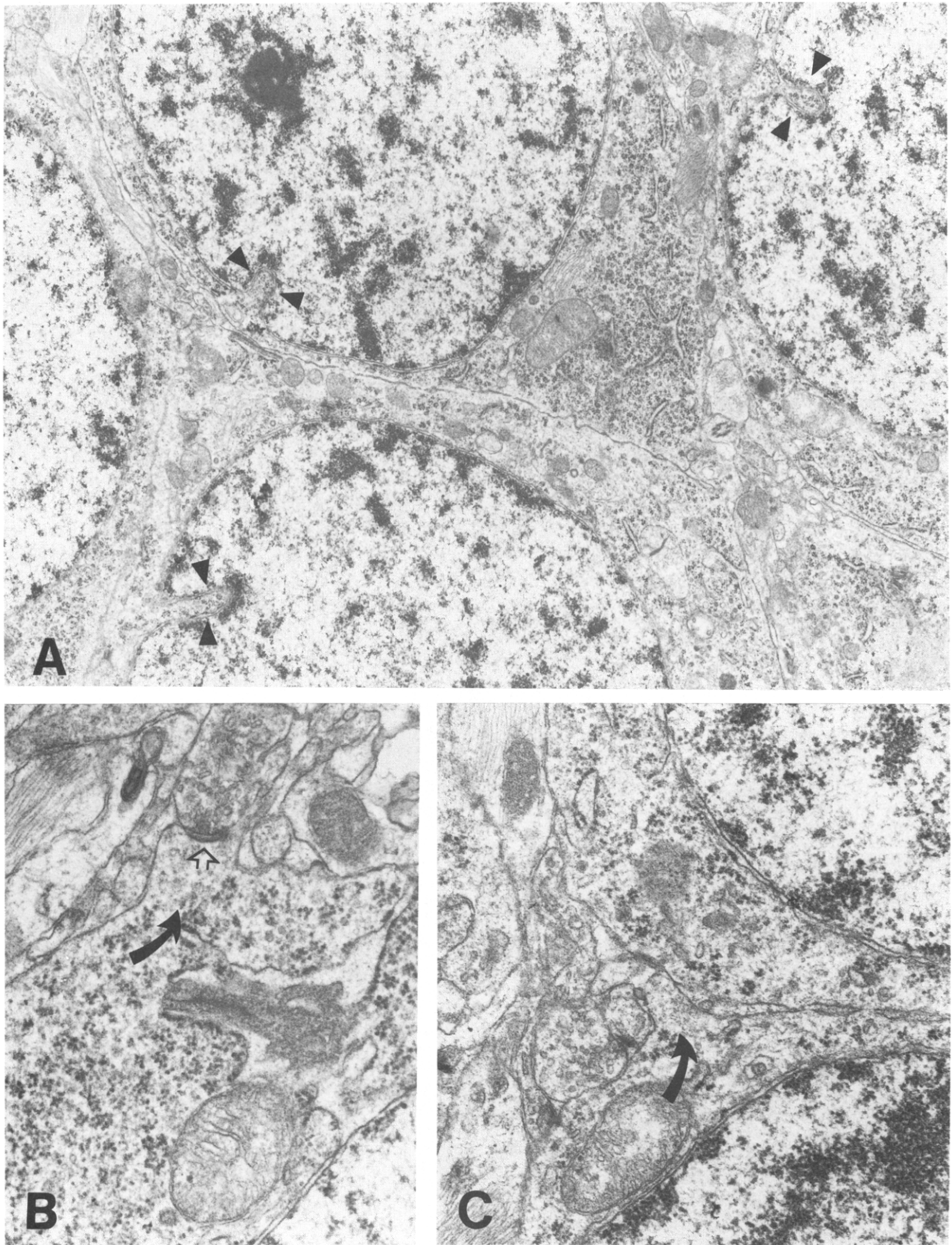


Fig. 2. Electron micrographs of granule cells in the monkey dentate gyrus. The nuclei of granule cells frequently display finger-like infoldings (arrowheads) of different sizes (A). Somal spines (curved arrows in B and C) appear regularly and they may be apposed by axon terminals that form synapses (open arrow in B). A,  $\times 13,500$ ; B,  $\times 39,000$ ; C,  $\times 32,000$ .

TABLE 1

Number of axosomatic synapses per granule cell body per thin section

	Total cells	Total synapses	Synapses/cell $\pm$ S.E.M.	Asymmetric synapses
Monkey 1	269	186	$0.691 \pm 0.048$	6
Monkey 2	404	260	$0.644 \pm 0.040$	5

axosomatic synapses found for those in adult rats<sup>15</sup>. This morphological difference between primate and rodent granule cells suggests a difference between the inhibitory control of these neurons in monkeys and rats because most of the terminals that form such synapses are derived from GABAergic inhibitory basket cells<sup>8,11</sup>. Another important source of inhibition of granule cells is derived from the chandelier cell that forms GABAergic inhibitory axonal plexuses with the axon initial segments of granule cells<sup>17</sup>. However, the present study did not analyze the axon initial segments of primate granule cells for synapses formed by this cell type.

The primate granule cells in the present study frequently displayed small nuclear infoldings whereas previous studies of granule cells in rats indicated that nuclear infoldings were rare<sup>7,8,14</sup>. A previous study of the primate dentate gyrus showed similar finger-like indentations of nuclear membranes of granule cells but the frequency of infoldings was not provided<sup>3</sup>. It is known that local circuit neurons in rats display multiple and deep nuclear infoldings<sup>8,15</sup> and have much greater physiological activity (fast-spiking cells) than granule cells in rats<sup>4</sup>. Therefore, the frequent appearance of nuclear infoldings for primate granule cells may indicate increased physiological activity.

Another feature commonly observed for the primate granule cell is the somal spine. In normal rats, somal spines are rare. However, mutant mice, transplanted

dentate gyrus and rats with induced seizures have granule cells that frequently display somal spines<sup>2,16,18</sup>. Since somal spines are correlated with periods of increased seizure activity, the presence of somal spines for primate granule cells may reflect increased physiological activity.

During the analysis of the ultrastructural features of granule cells, 3 interesting observations were made: a low number of symmetric axosomatic synapses and the presence of nuclear infoldings and somal spines. These 3 features of primate granule cells are different from those previously described for granule cells in rats. It is difficult to determine the actual link between these differences but the following discussion will attempt to show a connection to the physiological activity of granule cells.

Together, these 3 morphological differences between rat and monkey granule cells may indicate that primate granule cells are physiologically more active than rat granule cells. Another important anatomical feature that supports this notion is the presence of mossy fiber synapses with basal dendrites of granule cells<sup>10</sup>. Such a monosynaptic excitatory circuitry has not been shown for granule cells of normal rats. Thus, the nuclear infoldings and somal spines are probably morphological features that reflect higher physiological activity of granule cells in primates. The actual basis for this higher physiological activity may be derived from either decreased inhibitory input to granule cells based on the reduction in the number of symmetric axosomatic synapses or increased excitatory circuitry from the mossy fiber synapses with basal dendrites of primate granule cells. This hypothesized increased activity of granule cells in monkeys may make the primate dentate gyrus more susceptible to enter epileptiform bursting activity.

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